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THE GENETICS OF SUBSPECIFIC PHENOTYPE DIFFERENCES

IN *PIERIS OCCIDENTALIS* REAKIRT AND OF VARIATION
IN *P. O. NELSONI* W. H. EDWARDS (PIERIDAE)

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THE WESTERN WHITE, *Pieris occidentalis* Reakirt, has one of the widest latitudinal ranges of any nearctic butterfly—from near 36°N above 3000 m in the White Mountains of Mono and Inyo Counties, California to 69°N at sea level at Umiat, Alaska. The northernmost population to be studied biologically is located at Fairbanks, Alaska (64°49' N); its bionomics are described in Shapiro (1975a) and its phenotypic and developmental responses to photoperiod in Shapiro (1975b). This population corresponds to the taxon *Pieris nelsoni* W. H. Edwards (type locality St. Michael, Alaska), characterized phenotypically by a reduced black pattern dorsally in the male as compared with nominate *P. occidentalis* from California or Colorado (figs. 1, 2). *Pieris nelsoni* was described from a single male in 1883 and then "lost" for 91 years; during which time its female remained unknown along with its degree of relationship to *P. occidentalis*. Following its rediscovery at Fairbanks, *P. nelsoni* was reared in quantity for photoperiodism studies, which revealed a longer-day threshold for diapause induction and a reduced propensity to produce the estival phenotype (lightly marked ventrally) as compared with *P. occidentalis* (Shapiro, 1975b). However, its early stages were indistinguishable from *occidentalis* and the pattern of the female differed from that taxon only very subtly. These facts suggested strongly that *nelsoni* and *occidentalis* were geographic subspecies of a single species, an hypothesis tested by the crosses reported below.

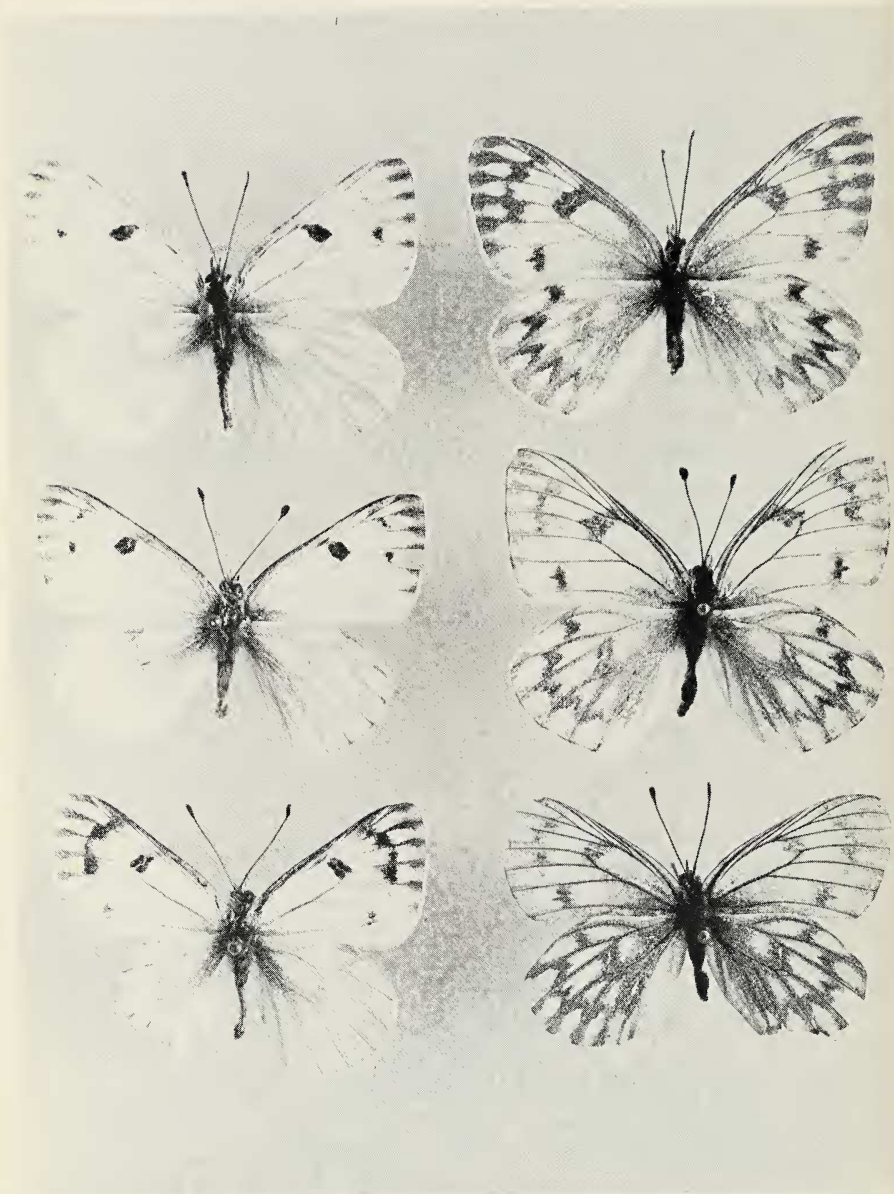


Fig. 1a.—Dorsal surfaces of wild *Pieris occidentalis nelsoni* from Fairbanks, Alaska, July 1974. Males at left.

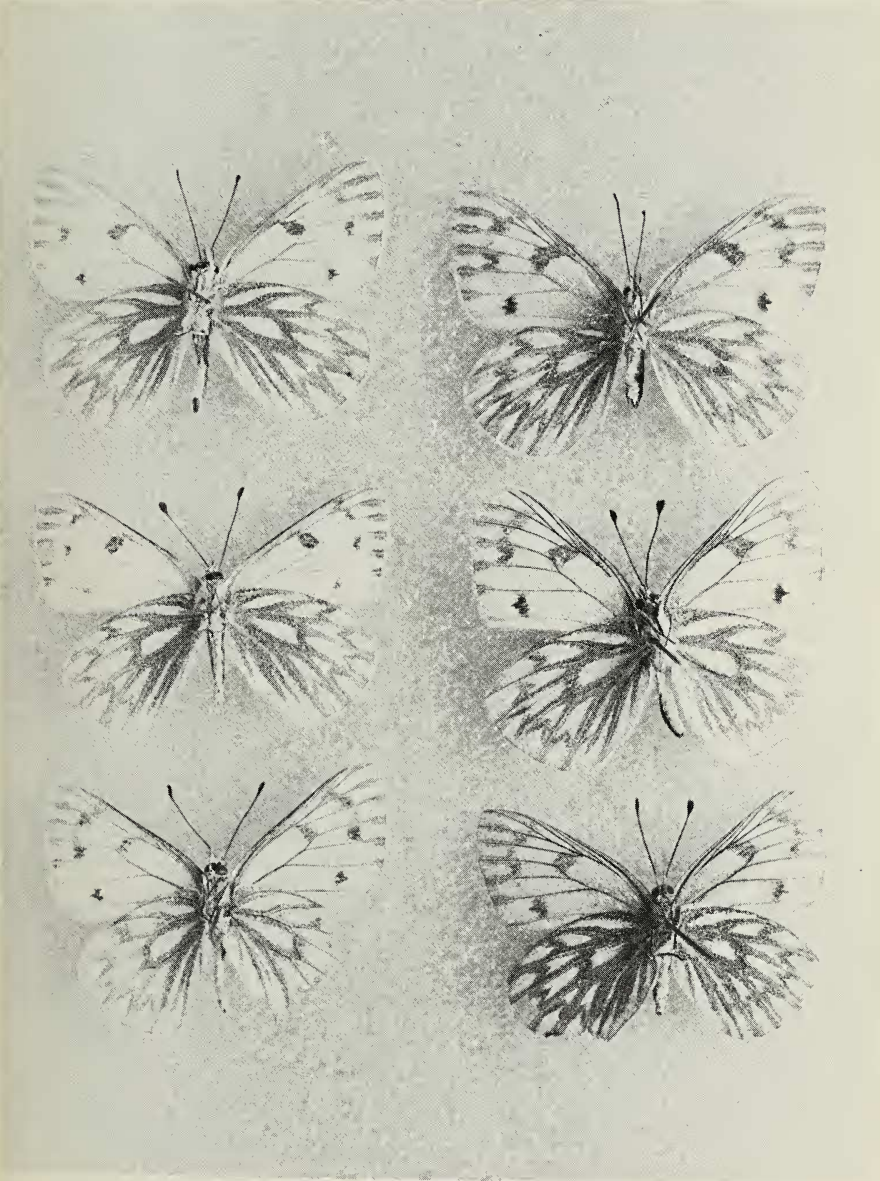


Fig. 1b.—Ventral surfaces of same.

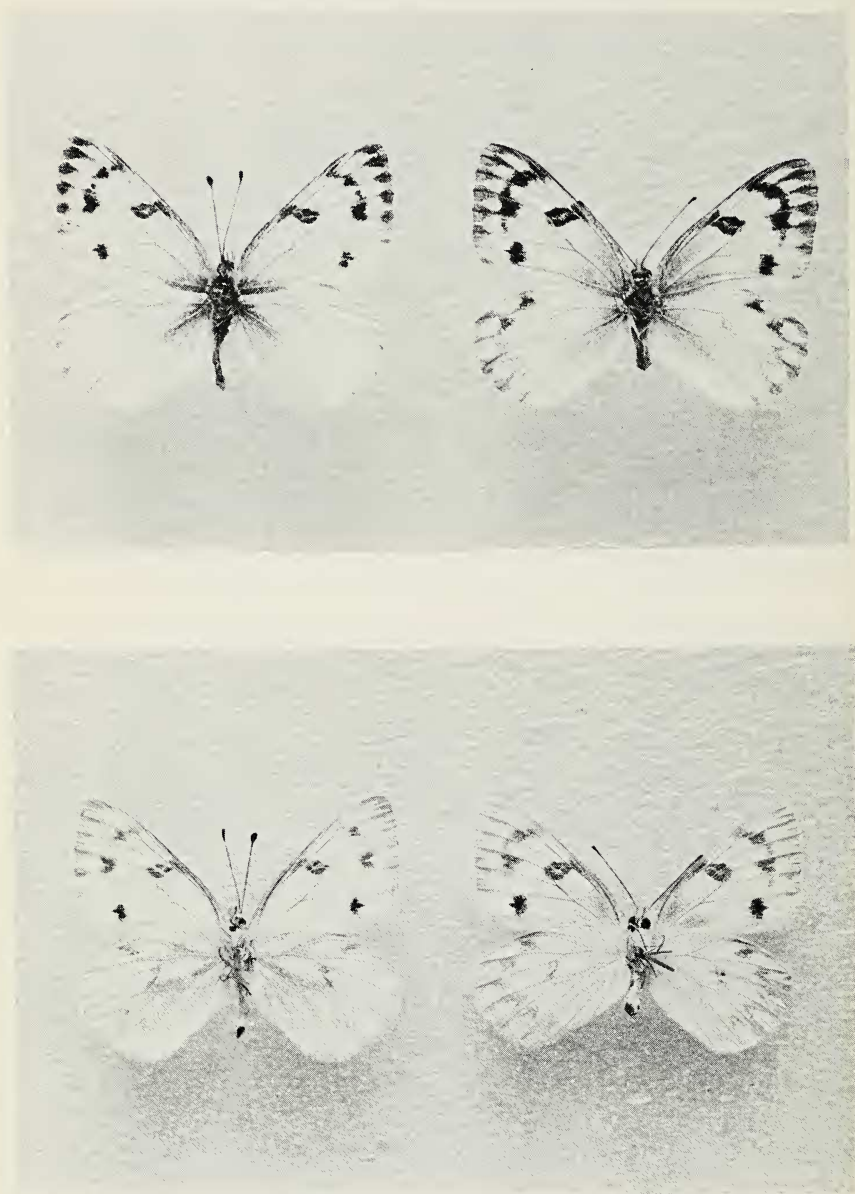


Fig. 2.—Dorsal and ventral surfaces of wild *Pieris occidentalis* from Donner Pass, California. Males at left.

In breeding *P. nelsoni* from Fairbanks I obtained a number of aberrant individuals which were mated and studied in continuous culture. These are described, figured, and discussed elsewhere in this paper.

MATERIALS AND METHODS

The initial cross of Alaskan and Californian stocks was made using a fresh wild male *P. occidentalis* collected at 3000 m at the base of Sonora Peak, Mono County, California on 8 August 1974 by S. R. Sims. This male was caged with two virgin female *P. nelsoni* of the first generation in continuous culture, ex Fairbanks. This stock was maintained at 25°C under continuous light and reared on fresh *Lepidium virginicum* L. (Cruciferae). The wild male was not very vigorous but did mate with one female on the second day, and this female laid 8 eggs, of which 5 hatched. All of these eventually pupated, but two subsequently died, producing an F₁ of only three individuals—two males and a female. A sib mating was, however, obtained, producing 49 ova of which 40 hatched, yielding 37 pupae and 25 adults of the F₂.

All rearing was done in colorless plastic Petri dishes (diameter 14 cm) on *L. virginicum* at densities of 10 larvae or less/dish at 25°C under a 60-W incandescent lamp. They pupated in the dishes, and only non-diapause pupae were obtained. Ecdysis took place in nylon-mesh cages 41 cm x 41 cm x 41 cm, and matings were obtained in these in filtered sunlight. All adults used for breeding were provided fresh dandelions as a nectar source. Mated females were confined in colorless plastic cages 19 x 13 x 10 cm with fresh cuttings of *Lepidium* and fresh dandelions and allowed to oviposit.

RESULTS OF HYBRIDIZATION

The F₁ consisted of two males, both intermediate between estival and vernal phenotypes, with complete *occidentalis* pattern, and one female with a dark-intermediate ventral pattern and a dorsal *occidentalis* pattern which, however, resembled *nelsoni* in having the spot at the end of the cell of the forewing narrow. All of these individuals became too battered to be figured.

The F₂ (figs. 3, 4) of 13 males and 12 females was exceedingly variable in all phenotypic characters, with seemingly independent assortment at several genetic loci. Within each sex, the



Fig. 3a.—Dorsal surfaces of F_2 males, Alaska X California *Pieris occidentalis*, reared at 25°C on continuous light.

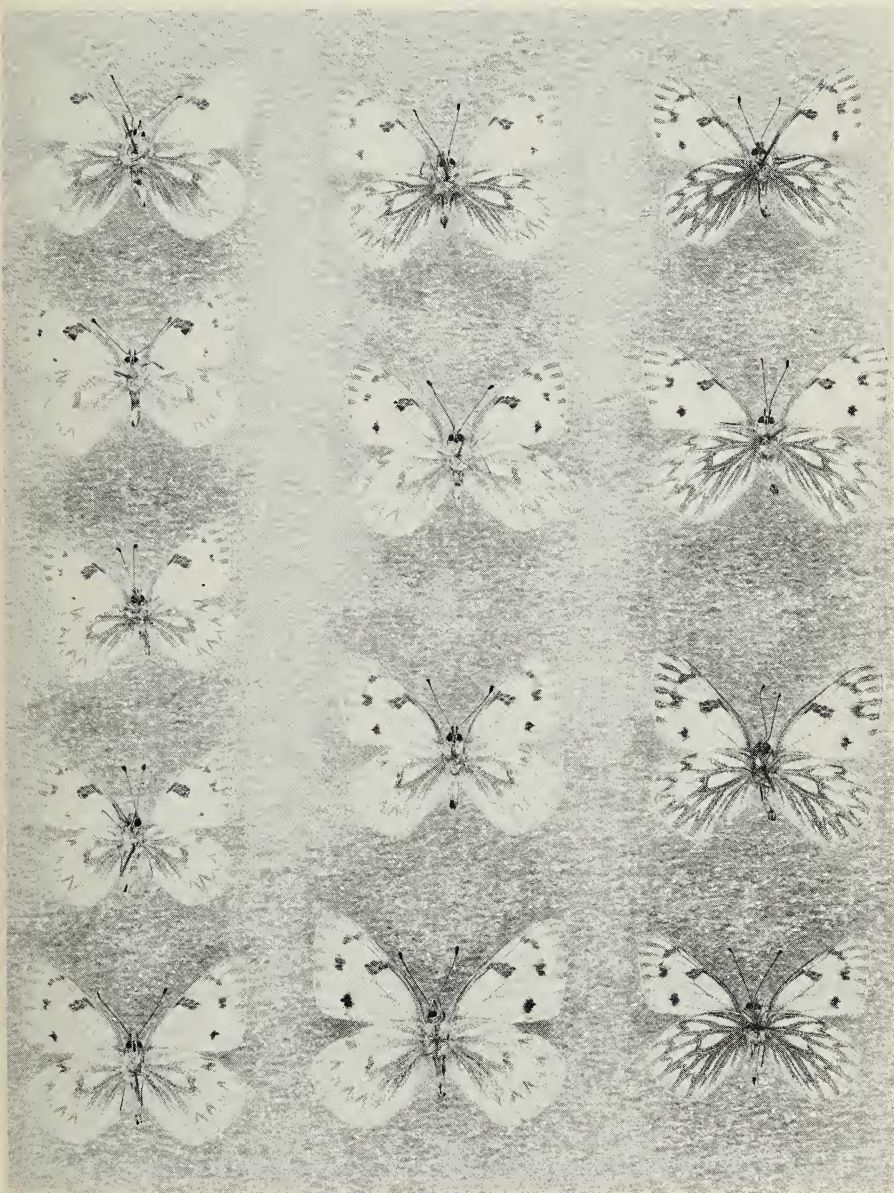


Fig. 3b.—Same but ventral.

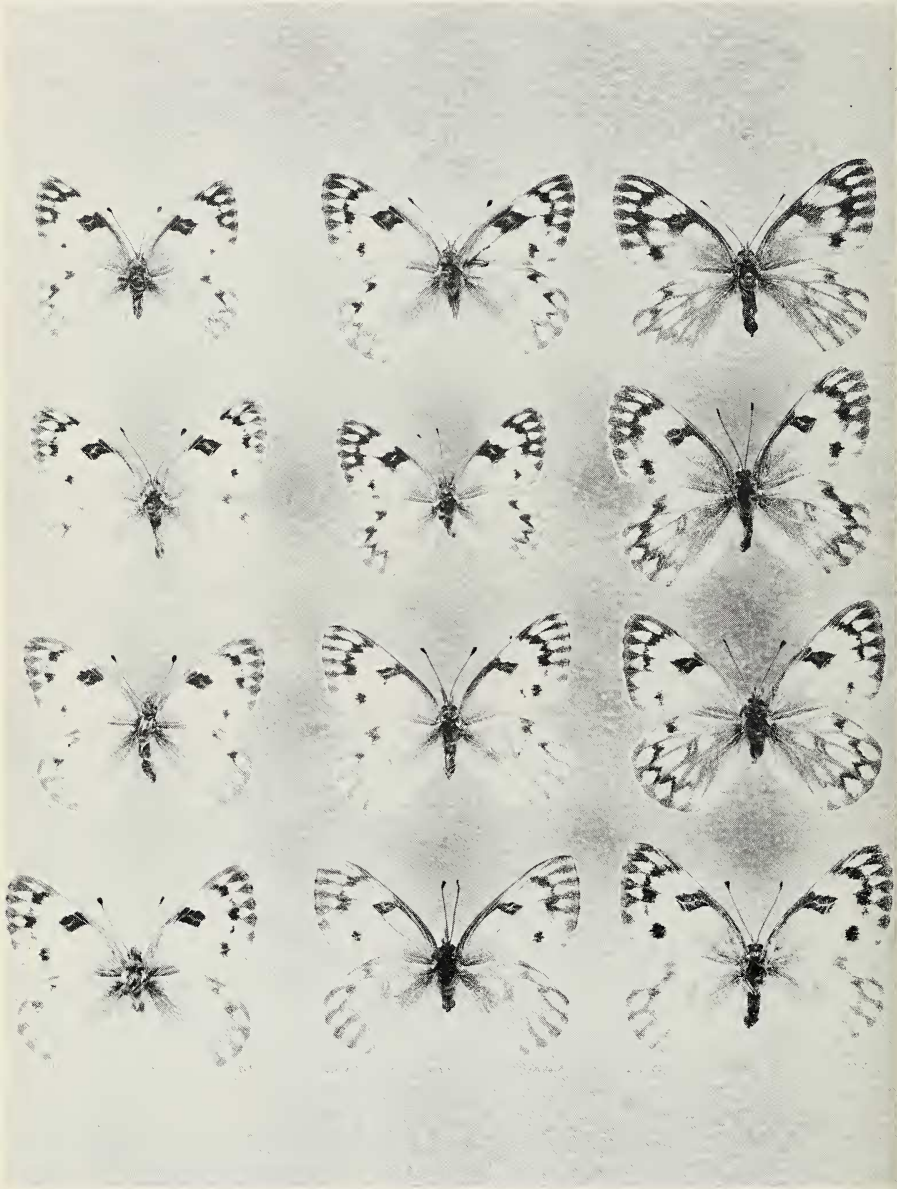


Fig. 4a.—Dorsal surfaces of F_2 females, Alaska X California *Pieris occidentalis*, reared at 25°C on continuous light.

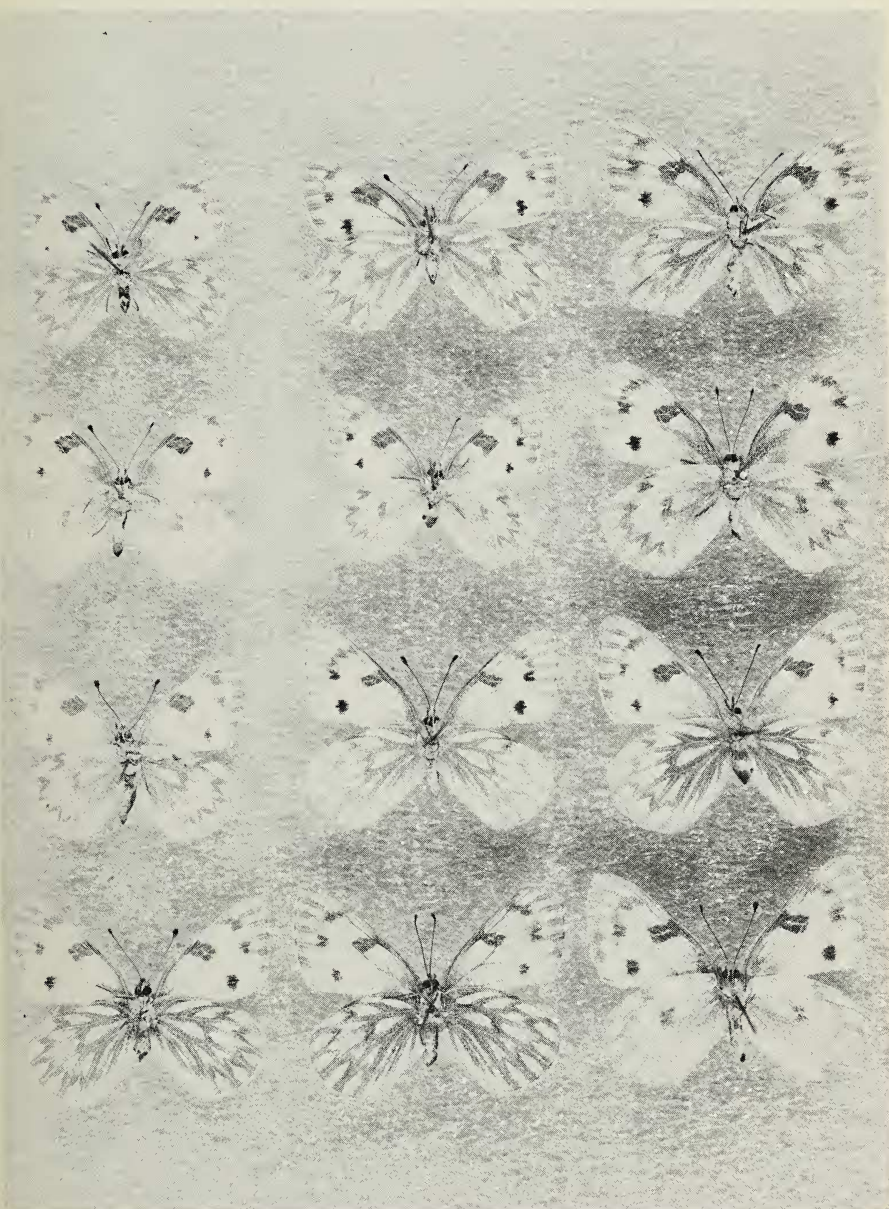


Fig. 4b.—Same but ventral.

phenotypic range includes apparently normal *occidentalis* and *nelsoni*, plus recombinants never seen in wild samples or pure bloods of either. Because of the small numbers of animals and the impracticability of large-scale genetic experimentation with these traits, only a very rough idea of their inheritance can be given. The traits which appear to be segregating are:

(1) *Dorsal pattern of male*. In *nelsoni*, the apical and sub-apical pattern elements are suppressed. This phenotype appeared only in one male of the F_2 , but several others had these black markings smaller than most male *occidentalis*. The sample is too small to discriminate between control by one locus, with the *nelsoni* allele recessive (expectation $1/4$ *nelsoni*), and two loci (expectation $1/16$ *nelsoni*).

(2) *Size and shape of spot in discal cell of forewing*. This trait is variable in both parental populations. The most frequent *nelsoni* state is "narrow" in both sexes; "narrow" is, however, rare in pure *occidentalis*. "Narrow" spot appeared in the F_1 female and in four males and four females of the F_2 . The males included the one with *nelsoni* dorsal pattern.

(3) *Dorsal pattern of female*. *Nelsoni* females do not differ from California *occidentalis* in any conspicuous character. They are more likely to have the black chevrons between the veins of the hindwing darker than the remainder of the pattern; the forewing more often has a complete (although narrow) black outer margin, and the white spots enclosed in the forewing border are usually broader in *nelsoni* than in *occidentalis*. The spot in cell Cu_2 is almost always smaller in female *nelsoni* and is occasionally lacking. In the F_2 , all of these characters are very variable. In three F_2 females and in the one F_1 female, the forewing border is broader than in either parental population, producing very elongate, narrow white spots enclosed within it. In five of the 12 F_2 females, the spot in cell Cu_2 is reduced as in *nelsoni*; in three it is heavier than in the average *occidentalis*. Both parental populations are variable as to basal dark shading, as is the F_2 . Control of female pattern is probably multifactorial and not an expression of the same major gene(s) involved in the male phenotypic difference.

(4) *Seasonal Phenotypes*. As noted in Shapiro, 1975b, *nelsoni* produces an estival phenotype (light ventrally), less often than does *occidentalis* when reared under continuous light. Again, the F_2 is extremely variable, and the variation is poorly correlated with characters on the dorsal surface. On the whole,

ventral melanization in both sexes in the F_2 is more similar to *nelsoni* than to Sierran *occidentalis*. In *nelsoni*, there is a tendency for the heaviest melanization to be basal, and this is found in four males and one female of the F_2 . One of the males is the single individual with dorsal *nelsoni* phenotype.

One F_2 female is asymmetrical. This female was not recognized as abnormal until after her death, but her phenotype is essentially identical to the trait "bilateral" previously found in Pennsylvania-New Jersey stock of the closely related species, *Pieris protodice* Boisduval and LeConte (Shapiro, 1970). This trait is inherited as an autosomal recessive. Some of the modifications produced in the homozygous "bilateral" *protodice* are related to the "ray" phenotypes discussed below.

ABNORMAL PHENOTYPES

The Fairbanks stock of *P. nelsoni* was started with five wild females, whose ova were pooled. In the F_1 , a number of aberrant individuals of both sexes were obtained, and several matings set up among them. The aberrant phenotype is apparently inherited as a simple autosomal dominant. It is expressed in both long- and short-day (estival and vernal) phenotypes (figs. 5, 6). Its differences from "wild type" are: Males: The submarginal black markings on the dorsal forewing are even more reduced than in "wild type" *nelsoni*, but the marginal triangles are present and may be slightly enlarged. The spot in cell Cu_2 is always absent and that at the end of the cell is slightly enlarged. Ventrally, the basal markings are more heavily melanized on the hindwing than "wild type" but the submarginal chevrons are only very weakly indicated. The spot at the end of the hindwing cell ventrally is enlarged. Females: The submarginal elements of the dorsal pattern are more or less reduced; as a minimum, the chevrons on the hindwing are not darker than the rest of the pattern and are thinner than in "wild type" *nelsoni*, making the enclosed white spots appear larger than normal; in estival-phenotype females the chevrons are usually obsolete. The spot at the end of the cell of the forewing is conspicuously enlarged (and ventrally may be prolonged basad along the cubitus, but not as a ray actually reaching the base). As in males, the basal melanization is exaggerated on the ventral hindwing.

In each of the six broods involving this phenotype reared in this study, a few highly abnormal individuals appeared in which the characters described above were exaggerated, along with distortion of the wing shape and (often) a reduction in

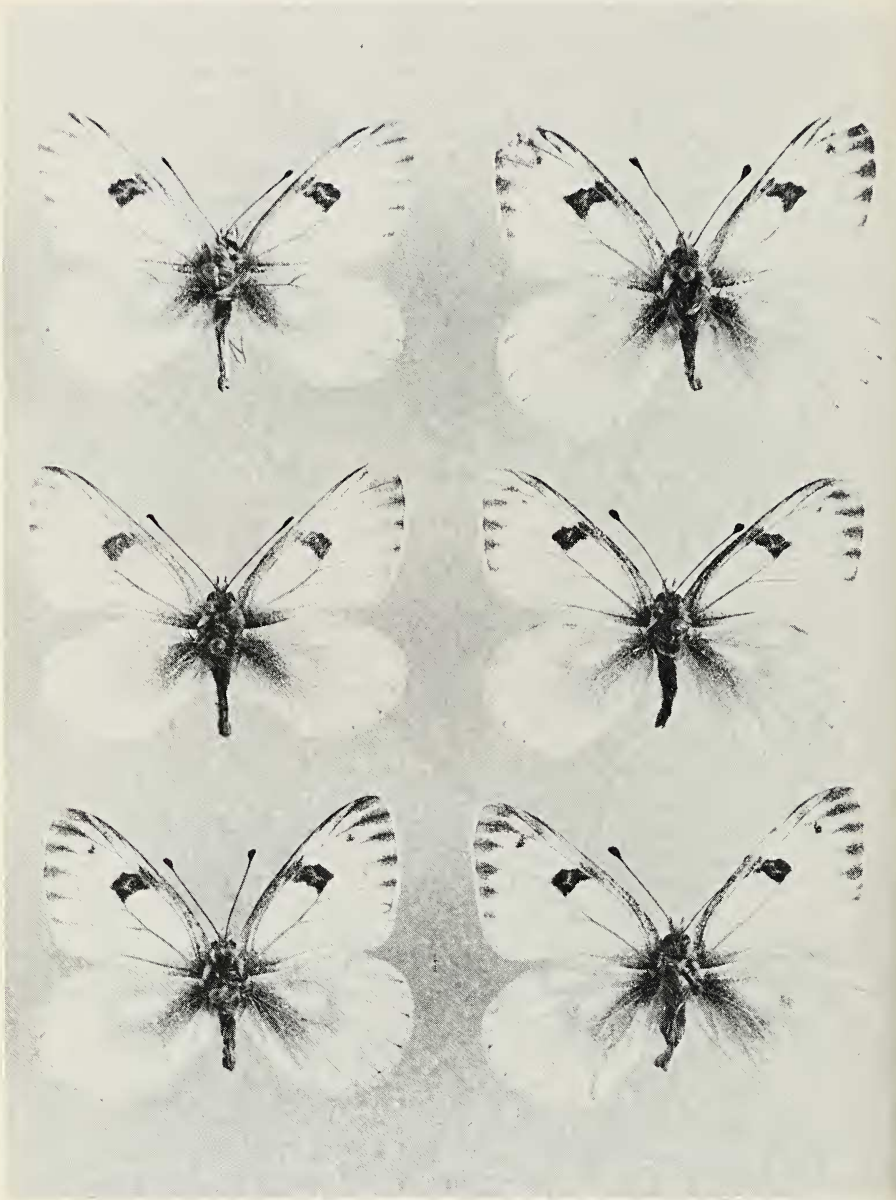


Fig. 5a.—Aberrant phenotypes of male *Pieris occidentalis nelsoni*, dorsal surfaces, illustrating light (estival) and dark (vernal) pattern.

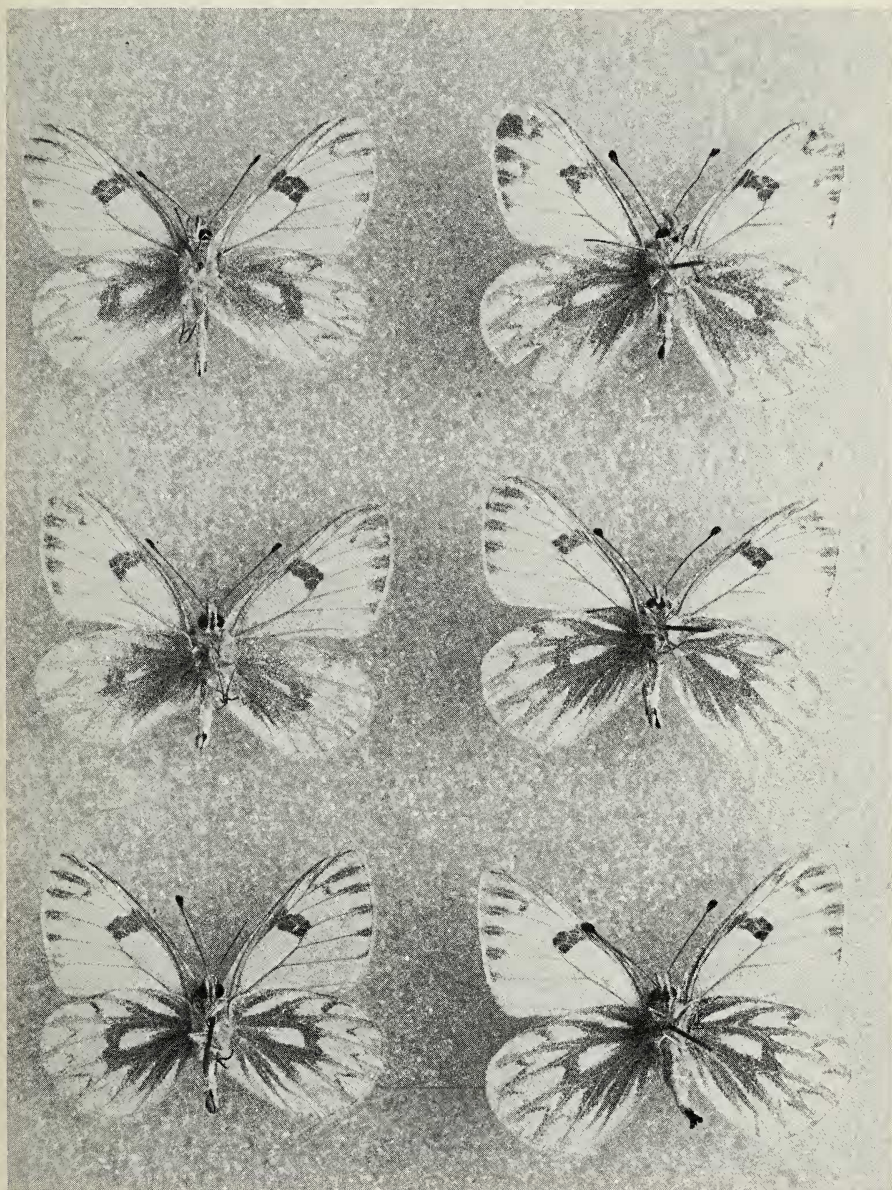


Fig. 5b.—Same but ventral.

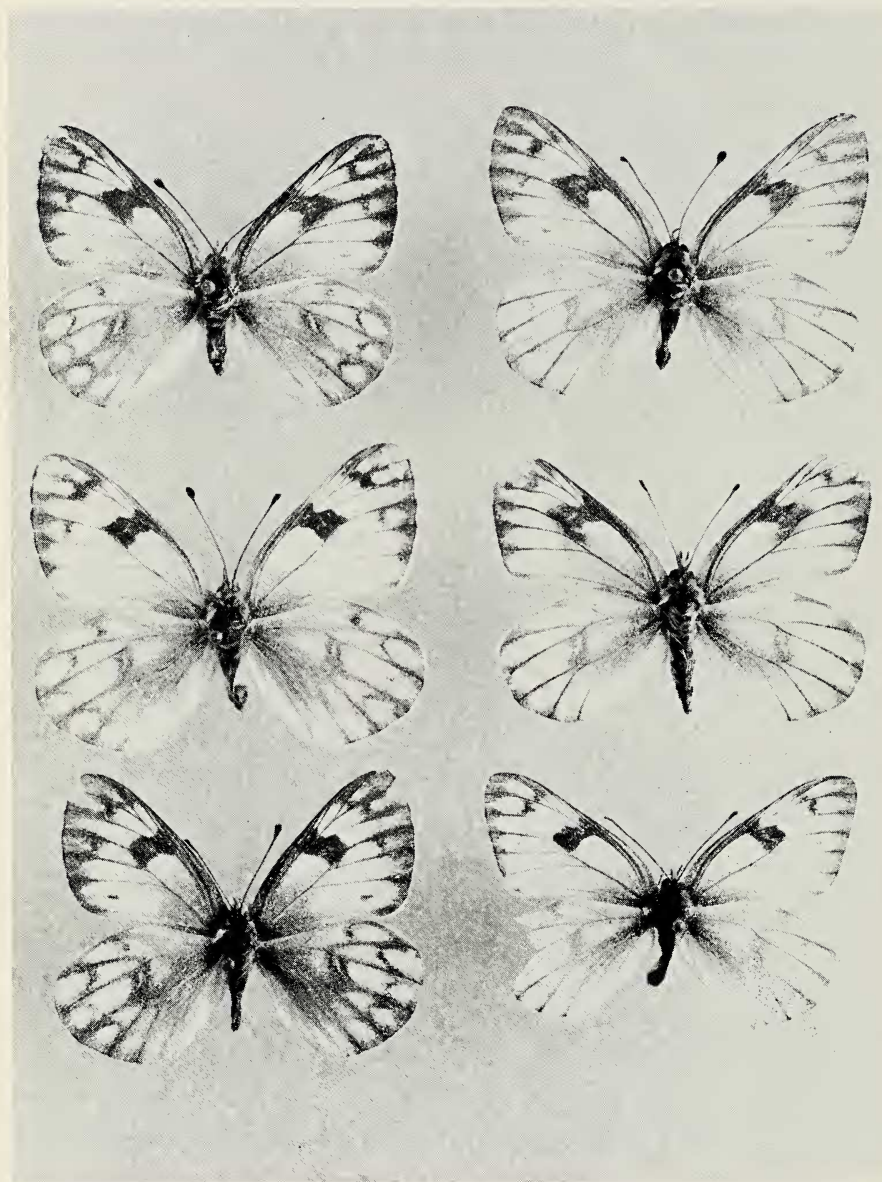


Fig. 6a.—Aberrant phenotypes of female *Pieris occidentalis neloni*, dorsal surfaces, illustrating light (estival) and dark (vernal) pattern.

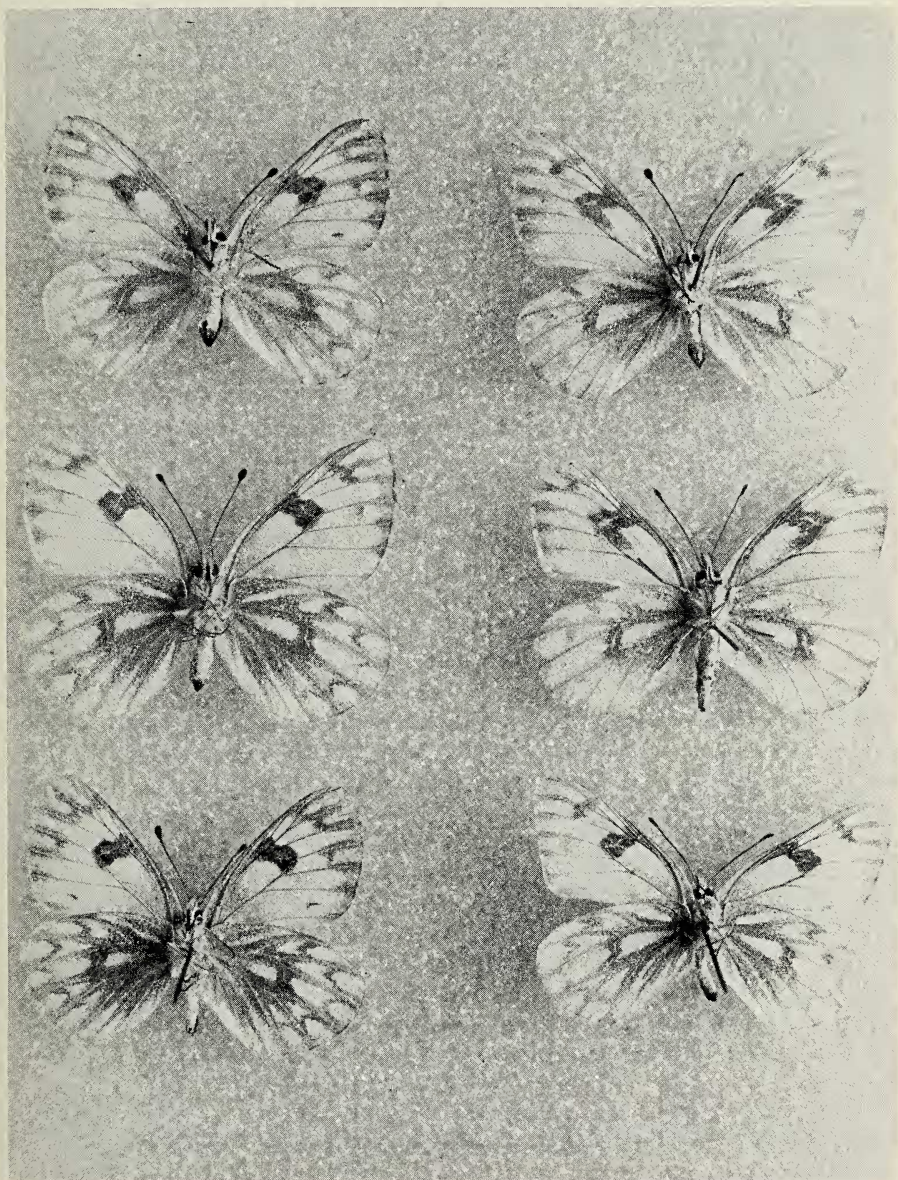


Fig. 6b.—Same but ventral.

the number of antennal segments. Some of these are illustrated in figures 7 and 8. These individuals were generally quite vigorous and lived 2-8 days, but most were unable to fly and all failed to mate, although the females were courted by normal males. These extreme phenotypes would presumably be lethal in Nature.

No wild specimens similar to those produced by this Fairbanks allele have been seen from any *Pieris occidentalis* population. Apparently, the male which mated with one of the founder females was heterozygous for it, but no examples were found among the 18 specimens collected at Fairbanks. The phenotype has some similarity to that of the mutant "ray" described from New York City stock of *P. protodice* by Shapiro (1973). The most extreme individuals produced in that line also somewhat resemble those reported here (fig. 9). However, "ray" is inherited as recessive. The dark "ray" along Cu on the forewing ventrally is perhaps produced in a variety of ways in this species group, as it occurs consistently (in females only) of Colorado *P. o. occidentalis* from above timberline and is inherited as a sex-limited dominant in crosses with Sierran stock (Shapiro, unpublished data).

Homozygous "ray" females often show stripping of scales along the outer margin below the forewing apices dorsally (Shapiro, 1973). This trait was observed in many Fairbanks specimens, both "wild type" and aberrant, and with equal frequency in both sexes. It may reflect adhesion of the wing to the pupal cuticle, such as occurs in the "albinensis" trait in *Pieris brassicae* Linnaeus (Gardiner, 1962). No difficulties with eclosion were observed in the pure Fairbanks line, but Gardiner was able to select for a line of *P. brassicae* in which "albinensis" eclosed normally, and the same selection may have occurred naturally at Fairbanks. When this trait was recombined with a California genome the system could have been disrupted, thereby contributing to the unusually high mortality of fully pigmented, ready-to-eclose pupae in the F₂. Only one adult which eclosed, the *nelsoni*-phenotype male, displays it.

The nature of the abnormalities observed in the Fairbanks material parallels those found in mass-reared stocks of *P. protodice* closely enough that one may assume similar developmental pathways are involved, whether or not the genetic control is homologous. Curiously, these abnormalities have not yet been seen in California *P. occidentalis*.

DISCUSSION

The hybridization experiments leave little doubt that *Pieris nelsoni* Edwards 1883 and *P. occidentalis* Reakirt 1866 are conspecific. By the International Code of Zoological Nomenclature, the former should be considered a subspecies of the latter, and written as *Pieris occidentalis nelsoni* (this combination was used by dos Passos, 1964). Left unresolved is the relation of both to *P. callidice* Hübner 1805, of the Palearctic region. This relationship can be clarified only by genetic experimentation.

The small size of the F_1 and F_2 Fairbanks x California broods precludes any convincing statistical analysis of the inheritance of phenotypic differences between the stocks, but it is evident that they must differ at several loci influencing color and pattern. In the case of differences in thresholds for diapause and phenotypic induction, these differences are clearly adaptive for the subarctic *nelsoni*. Even the aberrant phenotypes recovered from the Fairbanks stock would be potentially adaptive if the concentration of melanin at the hind wing base ventrally contributes to body warming during lateral basking. Strikingly, the reduction of the dorsal black pattern in the subarctic *nelsoni* parallels the appearance of extreme vernal phenotypes of *P. protodice* which fly in early spring in both eastern and western North America, suggesting an inherent advantage to this pigmentation under low-temperature conditions.

The hybrid broods are again too small for much to be said with confidence concerning the strength of postzygotic barriers, except that the fertility and viability of the F_2 did not depart significantly from many pure broods of both parental stocks when reared under high temperatures and continuous light. The sex ratio of the F_2 was normal. In butterfly hybrids involving genetic barriers there is usually a deficiency of females (the heterogametic sex). As noted previously, the high incidence of pupal mortality in the F_2 was not duplicated in the pure Fairbanks line but has been seen in some lots of Sierran pupae held at high temperatures in continuous culture; in these cases, however, most of the mortality occurs before adult pigment is laid down, while in the F_2 it was after.

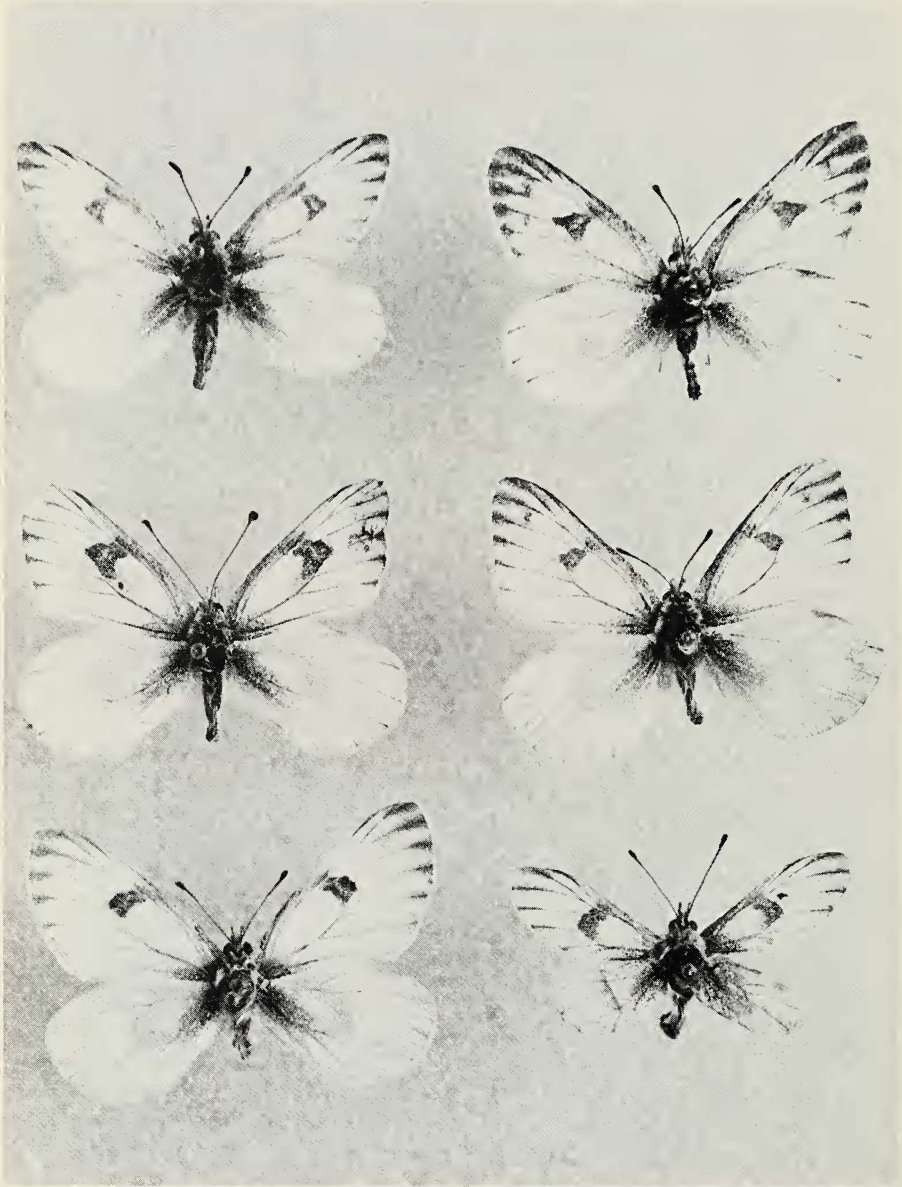


Fig. 7a.—Dorsal surfaces of extreme aberrant *Pieris occidentalis nelsoni* males.

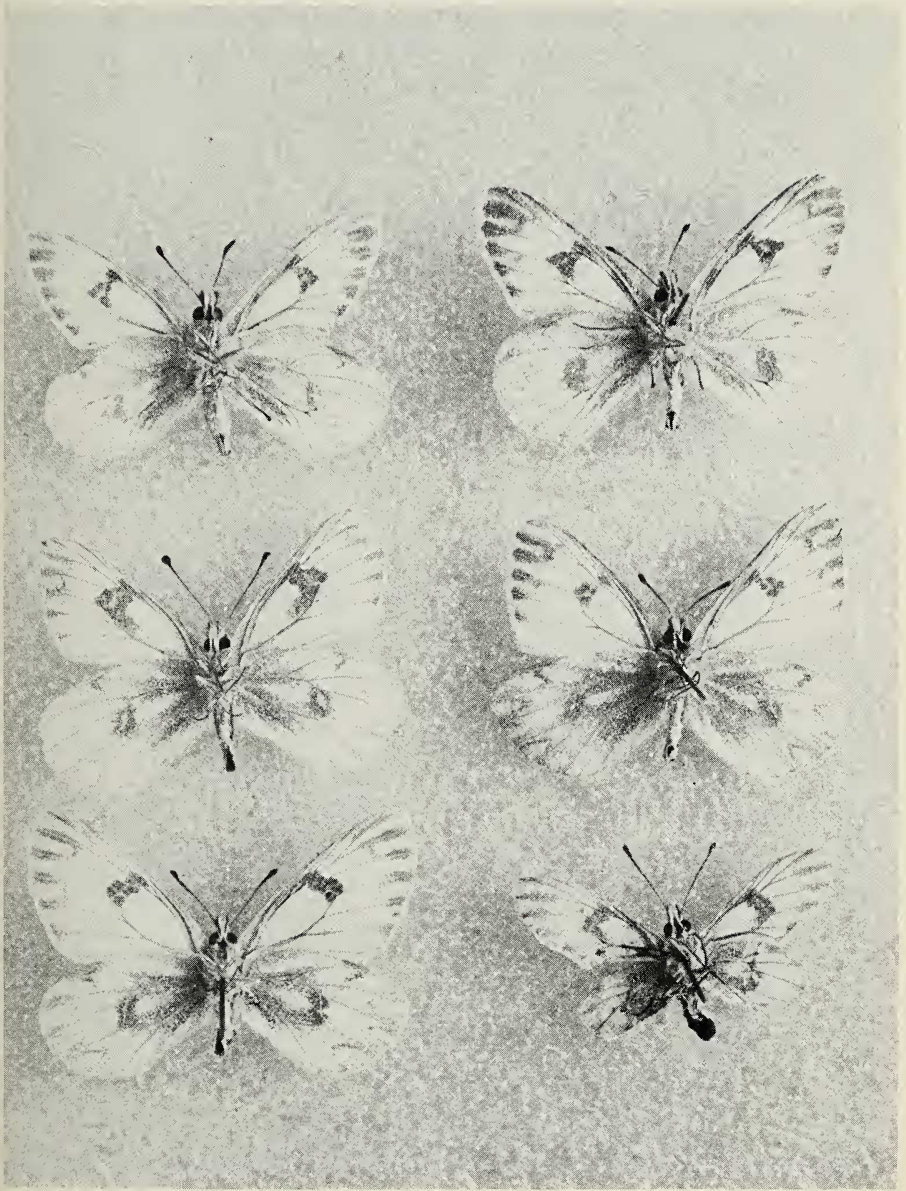


Fig. 7b.—Same but ventral.



Fig. 8a.—Dorsal surfaces of extreme aberrant *Pieris occidentalis nelsoni* females.

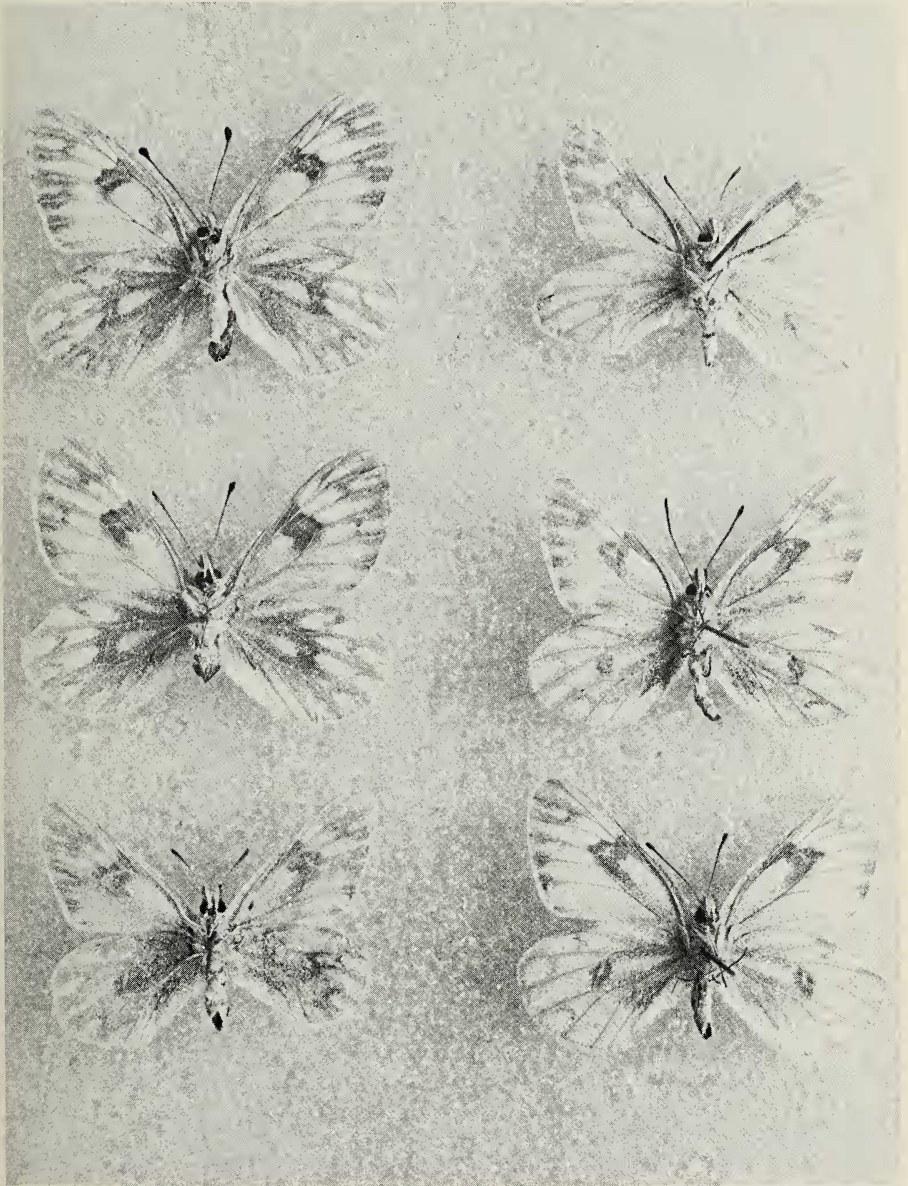


Fig. 8b.—Same but ventral.

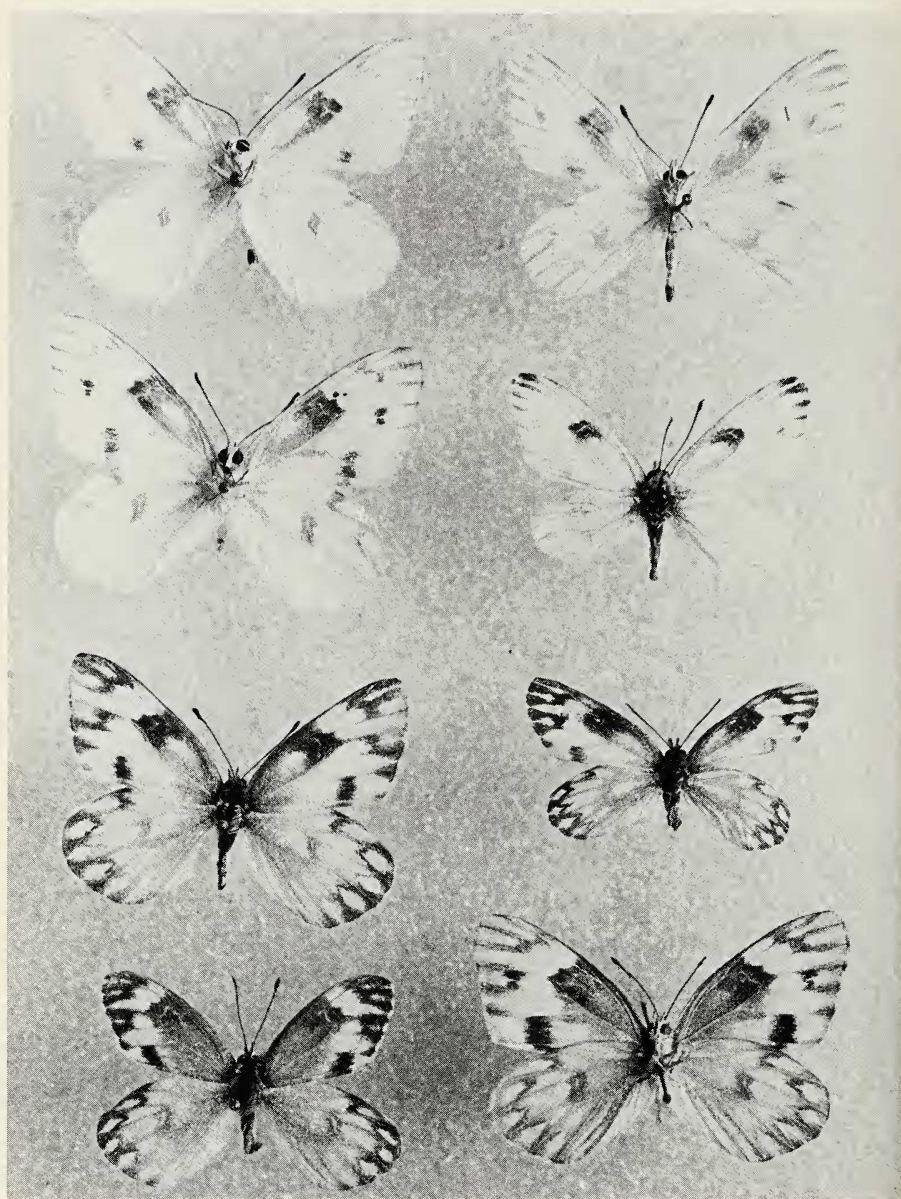


Fig. 9.—Selected examples of extremely aberrant *Pieris protodice* of the "Ray" line for comparison with Fairbanks stock of *P. o. nelsoni* (see Shapiro, 1973).

SUMMARY

1. Hybridization experiments demonstrate the apparent conspecificity of the taxa "*Pieris nelsoni*" from Alaska and *P. occidentalis* from the Sierra Nevada of California.
2. Three bred F₁ hybrids between these taxa were basically of the California phenotype, but within an F₂ of 25 individuals phenotypic variation was very great and recombination among several loci was evident.
3. An aberrant phenotype of *P. o. nelsoni* was obtained from the Fairbanks stock; it is inherited as an autosomal dominant. A few individuals exhibit functionally lethal exaggerations of the characteristics of this phenotype.
4. Aspects of the genetic variation of *P. o. nelsoni* are shown to parallel the related species *P. protodice*, which was studied previously.

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